Amendments to the Specification:

Please replace the instant Sequence Listing with new replacements sheets of the Sequence Listing attached herewith.

Please replace the title of the invention with the following amended title:

~~Production of recombinant fragments of muscle acetylcholine receptor and their use for ex-vivo immunoadsorption of anti-ACh receptor antibodies from myasthenic patients~~ METHOD OF REMOVING ANTI-AChR ANTIBODIES FROM THE SERUM OF A MYASTHENIA GRAVIS (MG) PATIENT USING A COMBINATION OF RECOMBINANT DOMAINS OF NICOTINIC ACETYLCHOLINE RECEPTOR (AChR) SUBUNITS

Please add the following new paragraph immediately after the title of the invention under new subsection title --Related Applications--:

This Application is the National Stage Entry of International Application No. PCT/GR03/00014, filed April 15, 2003, which claims the benefit of priority from Greece Application No. 20020100190, filed April 17, 2002.

Please replace the paragraph beginning on page 10, line 30, with the following amended paragraph:

Alpha 1-210 was enzymatically amplified by PCR using a full-length human muscle alpha cDNA clone. The upstream (5'-GCTGGCCTCGTCGAATTCTCCGAACATG-3') (SEQ ID N0:6) and downstream (5'-GATGAAGTAGAGGTCTAGACGCTGCATGACG-3') (SEQ ID N0:7) (primers were constructed to contain EcoRI and Xbal restriction sites (underlined). Using the appropriate restriction endonucleases, the purified cDNA fragment was subcloned into the expression vector pPICZalphaA (Invitrogen San Diego, CA) so that the recombinant fragment was led by a signal peptide alpha-factor under the transcriptional control of the AOX promoter, which is induced by methanol, while at the C terminal end it was fused to a sequence encoding the c-myc epitope and polyhistidine (6xHis) tag (FIG. 1A). The resulting construct was linearized using Pmel and transformed into the *Pichia pastoris* host strain GS115 by electroporation (BioRad GenePulser). Similar were the constructs used for the alpha1-80, 41-119 and 1-119, 1-211 polypeptides. The transformed cells were plated on YPDS (1% yeast extract, 2% peptone, 2% dextrose, and 1M sorbitol) plus zeocin (100 ug/ml) and incubated at 30°C for 3 days.

Please replace the paragraph beginning on page 13, line 6, with the following amended paragraph:

The DNA encoding the N-terminal extracellular domains of the human muscle nicotinic AChR beta, gamma and epsilon subunits was enzymatically amplified using PCR, on templates pcDNA-beta, pcDNA-gamma and pcDNA-epsilon respectively (plasmids carrying the whole cDNA of the gamma and epsilon subunits cloned under the CMV promoter) (plasmids provided by Dr Beeson, University of Oxford). PCR was performed using primers as follows. For beta subunit, upstream primer 5'-GCGGAATTCTCGGAGGCGGAGGG-TCGAC-3' (SEQ ID NO: 8) was combined with downstream primer 5'-ATAGTTTAGCGGCCGCTCAATGGTGATGGTGATGGTGCTTGCGGCG-GATGATGG-3' (SEQ ID N0:9) on template pcDNA-beta. For gamma subunit, upstream primer 5'- GGTGTAGAATTCCGGAACCAGGAGGAGCGC-3' (SEQ ID N0:10) was used with downstream primer ~~5'-ATAGTTTAGCGGCCGCTTAGTGATGGTGATGGTGATGCTTGCGCTGGATGAGC AGG-3'.~~ 5'-ATAGTTTAGCGGCCGCTTAGTGATGGTGATGGTGATGCTTGCGCTGGATGAGC AGG-3' (SEQ ID N0:11). For epsilon subunit, upstream primer 5'-GGTGTAGAATTCAAGAACGAGGACTGCG-3' (SEQ ID N0:12) was combined with downstream primer ~~5'-ATAGTTTAGCGGCCGCTTAGTGATGGTGATGGTGATGCTTCCGGCGGATGATG AGCGAG-3'.~~ 5'-

ATAGTTTAGCGGCCGCTTAGTGATGGTGATGGTGATGCTTCCGGCGGATGATG AGCGAG-3' (SEQ ID N0:13). All upstream primers were designed to contain an *Eco*RI site and the downstream primers were designed to contain a *Not*I site (these sequences are underlined in the shown primer sequences). All downstream primers contain a sequence which encodes for a six-histidine aminoacid tag to facilitate the purification of the expressed products (FIG. 1). For the expression of the subunits in the yeast *Pichia pastoris,* the obtained PCR fragments were digested with *Eco*RI and *Not*I enzymes, purified and cloned into the pPIC9 (Invitrogen, San Diego, Calif.) or pPIC9/FLAG vectors. The pic9/FLAG is a modification of the pPIC9 vector. This modified pPIC9/FLAG vector contains the FLAG amino acid sequence (DYKDDDDK) (SEQ ID NO: 14) immediately after the cleavage site of the secretion signal, which has been shown to increase solubility of the expressed polypeptide in *Pichia,* probably due to the highly hydrophilic nature of the amino acids. The modification was performed by cloning the oligo 5'-GTAGATTACAAGGATGACGATGACAAAG-3' (SEQ ID N0:15) between the unique *Sna*BI and *Eco*RI sites of the pPIC9 vector. This allows the subsequent cloning of any PCR product with an *Eco*RI site. The resulting plasmid was named pPIC9/FLAG.

Please replace the paragraph beginning on page 14, line 22, with the following amended paragraph:

The N-terminal extracellular domain of the human muscle AChR delta subunit (delta 1-224) was enzymatically amplified by RT-PCR using total RNA extracted from TE671 cells using the primers: 5'-GTGTGGCAGCGAATTCCTGAACGAG-3' (SEQ ID NO: 16) (upstream for delta subunit) and 5'-GATGTAGAATTCTCACTTGCGGCGG-3' (SEQ ID N0:17) (downstream for delta subunit). The primers were constructed to contain EcoRI restriction sites (underlined). Using the appropriate restriction endonucleases, the purified cDNA fragment was subcloned into the expression vector pPIC9, so that the recombinant fragment was led by a signal peptide alpha-factor under the transcriptional control of the AOX promoter, which is induced by methanol, while at the C terminal end there was no sequence encoding the c-myc epitope or the polyhistidine (6xHis) tag. The resulting construct was linearized using StuI and transformed into the *Pichia pastoris* host strain GS115 or into the *Pichia pastoris* clone that expressed alpha1-210 (example 1) in order to express delta1-224 alone or coexpressed together with alpha1-210 respectively. The transformed cells were plated on YPDS plus ampicillin (50 ug/ml). After induction for 3 days with daily addition of methanol (0.5% v/v), as described above, the culture supernatants were tested for expression of delta1-224 by dot-blot analysis using the anti-FLAG M2 monoclonal antibody (Sigma). The

clone with the highest protein yield was used for large-scale protein expression. The culture supernatant was precipitated using ammonium sulfate and dialyzed against TBS, pH 8, then delta1-224 was purified using Anti-FLAG M2 affinity gel (Sigma) according to the manufacturer's protocol, the recombinant protein being eluted under native conditions by competition with FLAG peptide (Sigma). The eluates were analyzed by 12% SDS-PAGE followed by Coomassie Brilliant Blue staining or Western blot analysis using anti-FLAG M2 monoclonal antibody. The yield of purified delta1-224 was estimated to 0.1-0.5 mg/l. The recombinant protein appeared as three bands of different molecular weight, which after in vitro deglycosylation of the product (as described above) migrated to the predicted from the amino acid sequence (26 kD). These results show that, like the native receptor delta subunit, delta1-224 is glycosylated. Delta1-224 was studied together with alpha1-210 in ^{125}I-alpha-BTX binding experiments. Results indicated a temporary weak interaction between the recombinant subunit domains alpha1-210 and delta1-224.

Please replace the paragraph beginning on page 17, line 5, with the following amended paragraph:

The DNA fragment encoding the extracellular domain of the human alpha AChR (alpha 1-210) was amplified by PCR using upstream primer 5'-GCGGCCGCATGAAGGTTCTGTGGGCTGCGTTGCTGGTCACATTCCTGGCAGGATGCCAGGCCTCCGAACATGAGACCCG-3' (SEQ ID NO:18) and downstream primer ~~5' CCGAGCCTCGAGTCAATGATGATGATGATGATGGTCGACG 3',~~ 5'-

CCGAGCCTCGAGTCAATGATGATGATGATGATGGTCGACG-3' (SEQ ID N0:19),

on template pTEP1-1. The primers provide the *Not*I and *Xho*I sites (underlined) for further manipulation of the DNA. Upstream primer also provides the leader sequence of the human apolipoprotein E (ApoE); this sequence encodes the recognizable tag on ApoE, which allows for extracellular secretion of the protein and therefore it might also allow secretion of our protein. Downstream primer encodes six additional histidine residues prior to the termination signal; the extra histidines will serve as a purification tool by use of nickel-agarose affinity chromatography. The obtained PCR product was digested using enzymes *Not*I and *Xho*I, filled in with the Klenow fragment of DNA polymerase I and cloned into *Sma*I site of the SFV-1 vector DNA.